ANTIOXIDANT AND DNA BINDING PARAMETERS OF N-ARYLHYDROXAMIC ACIDS

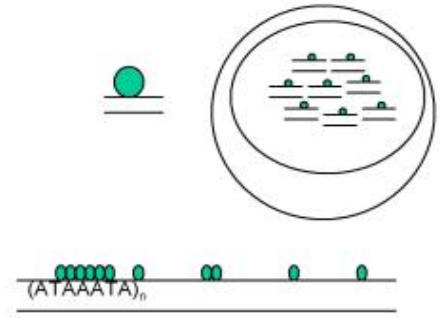
INTRODUCTION:

DNA is a crucial biological substance, the study on its action and detection has been becoming a main project in life science and pharmaceutical fields. The investigation of drug-DNA interaction is important to understand the molecular mechanism of drug action and for the design of specific DNA-targeted drug. The studies concerning the nature of interactions of small molecules with DNA are important not only for physical chemists but also for medicinal chemists. DNA plays a major role in the regulation of biochemical process and compounds capable of interacting with it, which exhibit a wide spectrum of biological activity, especially the antitumor one. The knowledge of binding interaction of small molecules with DNA is of interest for both therapeutic and scientific reasons (1-2).

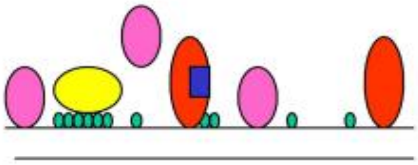
DNA binding interactions have been studied by several analytical methods includings electrophoresis (**3**), calorimetric titration (4), luminescence (5), UV absorption (6), fluorescence (7) and voltametric methods (8-12). Small molecules can interact with DNA in a variety of ways such as surface binding to their minor or major grooves, interaction between adjacent base pairs, covalent attachments to the double helix,or electrostatic binding.

The effect of DNA interacting Drugs within cells can be experimentally observed at many different levels.The first most basic level is the chemical interaction of the drug with the DNA double helix .

The Drug/ DNA interaction may stabilize a transient DNA structure, introducing a new conformation to DNA modification (13-15).



A. Direct DNA Interaction.

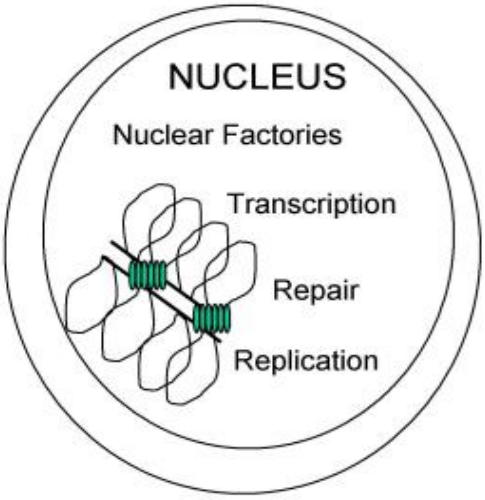


B. Nuclear Protein/DNA Interactions.

Binding

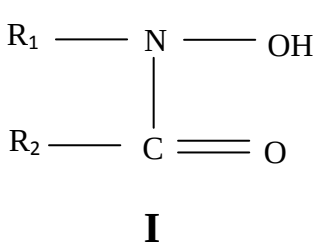
Displacement

Modification



C. Changes in Nuclear Metabolism and Structures.

In the present investigation, antioxidant behavior and the interaction of DNA with N-Aryihydroxamic acids will be experimentally measured for five molecules. N-Arylhydroxamic acids are the acyl/aryl derivatives of hydroxylamine or substituted hydroxylamine with general formula,



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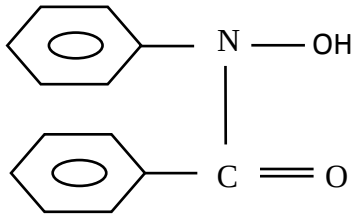


II

N-phenylbenzohydroxamic acid (PBHA)

PBHA is the parent molecule of this series and was first introduced by Bamberger in 1919 (16). Substitution of various groups at ortho, meta or para positions of upper and/or lower phenyl rings, gives a series of derivatives of PBHA.

In continuation of research work on “QSAR and Drug Designing” in our laboratory (17-18) , the binding interactions of PBHA and its analogous, with DNA will be studied following the UV, Fluorescence, Viscometric and Electrophoresis methods. For the first time the antioxidant behavior of N- arylyhydroxamic acids will be evaluated, following the Free Radical Scavenging, Carotene-Linoleate, and DNA Cleavage Methods (19).

A BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD AND NOTE WORTHY CONTRIBUTION IN THE FIELD OF PROPOSED WORK

N- Arylhydroxamic acids show biological activity as reported by this laboratory. Various QSAR parameters of these molecules have been determined and reported (20-21). The knowledge of binding interactions of DNA with N-arylhydroxamic acids is totally lacking. Therefore present investigation is undertaken.

PROPOSED METHODOLOGY DURING THE TENURE OF THE PRESENT WORK

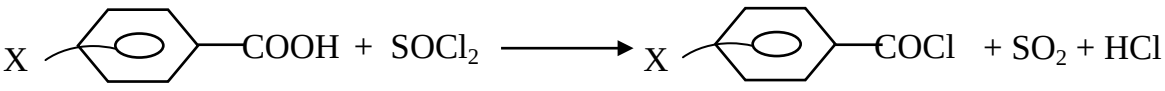
Five derivatives of N-arylhydroxamic acids will be synthesized following the procedure reported earlier (22-27). The methodology is proposed in the following sequence,

[A] SYNTHESIS OF HYDROXAMIC ACIDS

Synthesis of hydroxamic acids is a three step process.

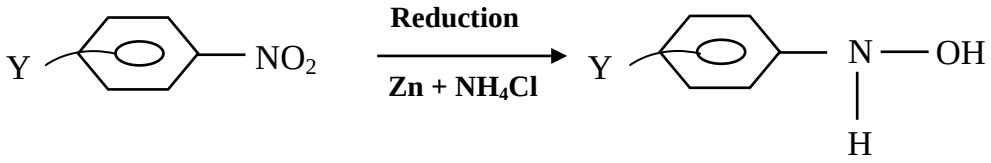
STEP I PREPARATION OF ACID CHLORIDE

Acid chloride will be prepared by the action of thionyl chloride on corresponding carboxylic acids.



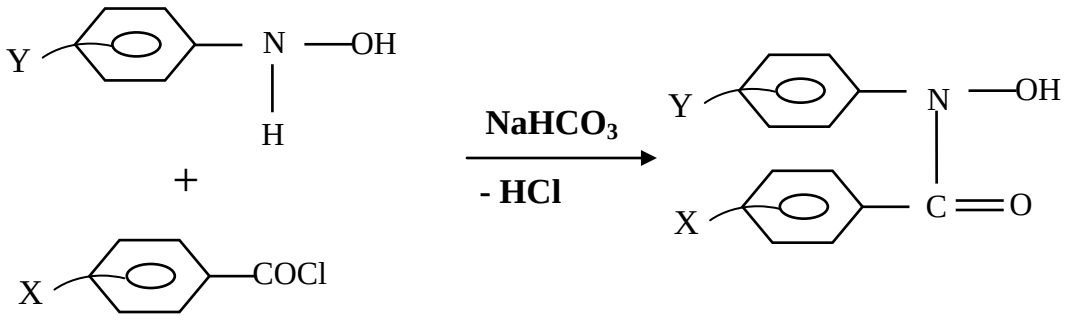
STEP II PREPARATION OF HYDROXYLAMINES

Hydroxylamine will be prepared by the reduction of corresponding nitro compounds in neutral medium.



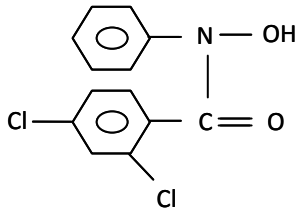
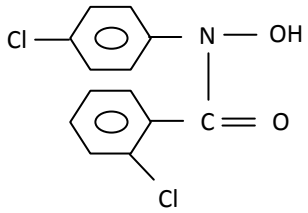
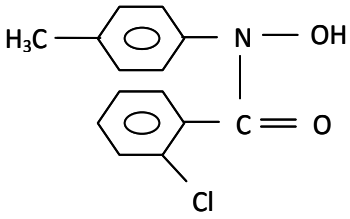
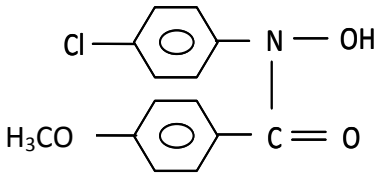
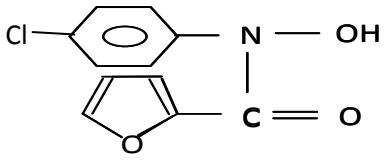
STEP III SYNTHESIS OF HYDROXAMIC ACIDS

Hydroxamic acids will be synthesized by coupling the hydroxyl amine with acid chloride in presence of sodium bicarbonate.



Where, X and Y are different substituent's and various position in upper or/and lower phenyl rings.

Following hydroxamic acids will be synthesized for the present investigation,

S.No.	Name of Compounds	Structure of Compounds
1.	N-phenyl-2-4 dichlorobenzohydroxamic acid	
2.	N-p-chlorophenyl-o-chloro benzohydroxamic acid	
3.	N-p-toly-o-chloro benzohydroxamic acid	
4.	N-p-chlorophenyl-p-methoxybenzohydroxamic acid	
5.	N-p-chlorophenyl-2-furohydroxamic acid	

[B] DNA BINDING INTERACTIONS

The anti-cancer activity and apoptosis studies of these reagents have been reported by this laboratory.(28) However it has not been investigated that these reagents have DNA binding and cleavage properties.

The binding interactions will be measured by following methods.

I. UV-ABSORPTION STUDIES

UV- absorption is one of the method to investigate the binding of a molecule with DNA. The UV-absorption spectroscopy experiments will be conducted by adding a fix amount of DNA solution to its sample of hydroxamic acids (HA) at different concentrations(C). The pH around 7.0 will be adjusted using buffer. The absorption spectra will be recorded in the absence and presence of DNA.

The binding constants will be calculeted following the equation (29-30).

$$\frac{DNA}{\varepsilon_a - \varepsilon_t} = \frac{DNA}{\varepsilon_b - \varepsilon_t} + \frac{1}{K_b(\varepsilon_b - \varepsilon_t)} \qquad [1]$$

where,

DNA	=	Concentration of DNA.
$\varepsilon_{a,}$	=	Apparent extinction coefficients.
	=	$(A_{obs}/[M])$.
ε_t	=	Extinction coefficients of ligand.
ε_t	=	Extinction coefficients of ligand-DNA complex in bound form.
K_b	=	Binding constant.

Procedure: The spectrophotometric titration will be carried out at room temperature to determine the binding affinity between DNA and hydroxamic acids. The spectrum of DNA-HA complex as sample and buffer will be recorded in UV region . The buffered DNA solution will be used to eliminate the absorbance of DNA itself. The procedure will continue, until there will be no change in spectra. This will indicate the attainment of binding saturation (31). Many anticancer drugs work by targeting DNA within the nucleus of cells (32). These drugs may exert their primary clinical effects by interfering with the template function of DNA either by blocking gene transcription or inhibiting DNA replication.

The exact mode of action for hydroxamic acids is not defined. Therefore it is necessary to gain the knowledge of interactions between small molecules and DNA, specially at the site mode, sequence and structural specificity of binding reactions.

II. BY VISCOSITY MEASUREMENT

Viscosity measurements give valuable information regarding the mode of binding of a molecule with DNA.

Procedure : Sonificated DNA solution of known concentration in DMSO will be placed in viscometer. The sample solution in DMSO will be directly added to this solution after fixed intervals and flow time will be measured after each addition. The relative viscosity will be calculated following the equation (33).

$$\eta / \eta_0 = (t-t_0)/(tDNA-t_0) \quad [2]$$

where,

t_o = Flow time of buffer.

t_{DNA} = Flow time of buffer containing DNA .

t = Flow time of buffer containing DNA after the addition of HA.

III. FLUORESCENCE SPECTRA : FQ METHOD

The efficiency of quenching of flurophore by quenching species follows Stern-Volmer relationship(34),

$$F_0/F = 1+Ks[Q] \quad [3]$$

where,

F_0 = Fluorescence intensities in absence of quencher.

F = Fluorescence intensities in presence of quencher.

Ks = Stern-Volmer quenching constant.

Q = Concentration of quenchers.

[C] DETERMINATION OF ANTIOXIDANT PROPERTY,

The following three methods will be applied to measure the antioxidant property,

1 Free Radical Scavenging Method

2 β-Carotene-Linoleate Method

3 DNA Cleavage Method.

I. FREE RADICAL SCAVENGING METHOD

This is the most widely reported method for screening of antioxidant activity of many plant drugs. DPPH assay method is based on the reduction of ethanolic solution of colored free radical DPPH by free radical scavenge. DPPH is a common abbreviation for an organic chemical compound 1,1-diphenyl-2-picryl-hydrazyl. It is a dark-colored crystalline powder composed of stable free-radicals. The antioxidant activity of the compound is expressed as (35-37)

$$\% \text{ Inhibition} = [(A_0 - A_S / A_0) * 100] \quad [4]$$

where,

A_0 = Absorbance of the blank solution

A_S = Absorbance of the sample solution.

II. β-CAROTENE-LINOLEATE METHOD

The rate of β-carotene bleaching for PBHA, will be calculated according to first-order kinetics (38-40),

$$R = 1/t \times \ln A_0/A_t \quad [5]$$

where, t = time in minutes

A_0 = initial absorbance

A_t = absorbance after time interval.

Based on the rates determined at three time intervals, the average rate of β-carotene degradation can be calculated. Furthermore, the antioxidant activity will be calculated from the absolute changes in absorbance. The first offers no protection against oxidation of β-carotene in emulsion, while the other offers maximum protection during the assay. Accordingly, the antioxidant activity of the compound is expressed as [6]

$$\mathbf{AA = \left[1 - \frac{R_t}{R_0}\right] x\ 100} \qquad \mathbf{[6]}$$

where,

R_t = rate of inhibition with sample

R_0 = control rate of inhibition.

III. DNA CLEAVAGE METHOD

For the gel electrophoresis experiment, pBR322 supercoiled plasmid DNA was treated with the hydroxamic acids in Tris-acetate buffer of pH 7.4 at room temperature. The samples were analyzed by electrophoreses for 1 hour at 100V in Tris-acetate buffer containing 1% agarose gel. Degerative diseases caused by oxidative form of DNA. (41).

EXPECTED OUTCOME OF THE PROPOSED WORK

DNA is the molecule of great biological significance. The total DNA content of a cell is known as GENOME. It is unique to an organism, and is the information bank governing all life processes of the organism.

The drug can interact with DNA in a variety of ways as follows,

I) Surface binding to their major or minor grooves.

II) Intercalation between adjacent base pairs.

III) Covalent attachment to the double helix.

IV) Electrostatic binding.

Hydroxamic acids are drug like molecules which show anti-tumor/anti-cancer activity. The knowledge of their DNA-binding interactions and antioxidant property will be helpful to design a better product.

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